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HBV variants detection and application

BACKGROUND OF THE INVENTION**5 FIELD OF THE INVENTION**

The present invention relates generally to viral variants exhibiting reduced sensitivity to particular agents and/or reduced interactivity with immunological reagents. More particularly, the present invention is directed to hepatitis B virus (HBV) variants exhibiting
10 complete or partial resistance to nucleoside or nucleotide analogs and/or reduced interactivity with antibodies to viral surface components including reduced sensitivity to these antibodies. The present invention further contemplates assays for detecting such viral variants, which assays are useful in monitoring anti-viral therapeutic regimens and in developing new or modified vaccines directed against viral agents and in particular HBV
15 variants. The present invention also contemplates the use of the viral variants to screen for and/or develop or design agents capable of inhibiting infection, replication and/or release of the virus.

DESCRIPTION OF THE PRIOR ART

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Bibliographic details of the publications referred to in this specification are also collected at the end of the description.

The reference to any prior art in this specification is not, and should not be taken as, an
25 acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in any country.

Hepatitis B virus (HBV) can cause debilitating disease conditions and can lead to acute liver failure. HBV is a DNA virus which replicates *via* an RNA intermediate and utilizes
30 reverse transcription in its replication strategy (Summers and Mason, *Cell* 29: 403-415, 1982). The HBV genome is of a complex nature having a partially double-stranded DNA

structure with overlapping open reading frames encoding surface, core, polymerase and X genes. The complex nature of the HBV genome is represented in Figure 1. The polymerase consists of four functional regions, the terminal protein (TP), spacer, reverse transcriptase (rt) and ribonuclease (RNase).

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The polymerase gene of HBV overlaps the envelope gene, mutations in the catalytic domain of the polymerase gene can also affect the nucleotide and the deduced amino acid sequence of the envelope protein and *vice versa*. In particular, the genetic sequence for the neutralization domain of HBV known as the 'a' determinant, which is found within the HBsAg and located between amino acids 99 and 169, actually overlaps the major catalytic regions of the viral polymerase protein and in particular domains A and B.

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The presence of an HBV DNA polymerase has led to the proposition that nucleoside or nucleotide analogs could act as effective anti-viral agents. Examples of nucleoside or nucleotide analogs currently being tested are penciclovir and its oral form (FCV) [Vere Hodge, *Antiviral Chem Chemother* 4: 67-84, 1993; Boyd *et al.*, *Antiviral Chem Chemother*. 32: 358-363, 1987; Kruger *et al.*, *Hepatology* 22: 219A, 1994; Main *et al.*, *J. Viral Hepatitis* 3: 211-215, 1996], Lamivudine [(-)- β -2'-deoxy-3'-thiacytidine]; (3TC or LMV) [Severini *et al.*, *Antimicrobial Agents Chemother*. 39: 430-435, 1995; Dienstag *et al.*, *New England J Med* 333: 1657-1661, 1995]. New nucleoside or nucleotide analogs which have already progressed to clinical trials include the pyrimidines Emtricitabine, ((-)- β -L-2'-3'-dideoxy-5-fluoro-3'-thiacydidine; FTC), the 5-fluoro derivative of 3TC, and Clevudine (1-(2-fluoro-5-methyl- β -L-arabino-furanosyl) uracil; L-FMAU), a thymidine analog. Like 3TC, these are pyrimidine derivatives with an unnatural "L"- configuration.

25 Several purine derivatives have also progressed to clinical trials; they include Entecavir (BMS-200, 475; ETV), a carbocyclic deoxyguanosine analog, diaminopurine dioxolane (DAPD), an oral pro-drug for dioxolane guanine ((-)- β -D-2-aminopurine dioxolane; DXG) and Adefovir dipivoxil, an oral prodrug for the acyclic deoxyadenosine monophosphate nucleoside or nucleotide analog Adefovir (9-[phosphonyl-methoxyethyl]-adenine; PME). Other drugs in pre-clinical and clinical trials include FLG [Medivir], ACH-126,443 (L-d4C) [Archillion Pharmaceuticals], ICN 2001-3 (ICN) and Racivir (RCV) [Pharmasset].

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Whilst these agents are highly effective in inhibiting HBV DNA synthesis, there is the potential for resistant mutants of HBV to emerge during long term antiviral chemotherapy. In patients on prolonged LMV therapy, key resistance mutations are selected in the rt domain within the polymerase at rtM204I/V +/- rtL180M as well as other mutations. The nomenclature used for the polymerase mutations is in accordance with that proposed by Stuyver *et al.*, 2001, *supra*. LMV is a nucleoside or anucleotide analog that has been approved for use against chronic HBV infection. LMV is a particularly potent inhibitor of HBV replication and reduces HBV DNA titres in the sera of chronically infected patients after orthotopic liver transplantation (OLT) by inhibiting viral DNA synthesis. LMV monotherapy seems unlikely to be able to control HBV replication in the longer term. This is because emergence of LMV-resistant strains of HBV seems almost inevitable during monotherapy.

Adefovir dipivoxil (ADV: formerly, bis-pom PMEA) is an orally available prodrug of the acyclic deoxyadenosine monophosphate analog adefovir (formerly, PMEA) (Figure 2). ADV is also a potent inhibitor of HBV replication and has recently been given FDA approval for use against chronic HBV infection. Adefovir dipivoxil differs from other agents in this class in that it is a nucleotide (vs. nucleoside) analog and as such bypasses the first phosphorylation reaction during drug activation. This step is often rate-limiting. Adefovir dipivoxil has demonstrated clinical activity against both wild-type and lamivudine-resistant strains of HBV and is currently in phase III clinical Testing (Gilson *et al.*, *J Viral Hepat* 6: 387-395, 1999; Perrillo *et al.*, *Hepatology* 32: 129-134, 2000; Peters *et al.*, *Transplantation* 68: 1912-1914, 1999; Benhamou *et al.*, *Lancet* 358: 718-723, 2001). During phase II studies a 30 mg daily dose of adefovir dipivoxil resulted in a mean 4 log₁₀ decrease in viremia over 12 weeks (Heathcote *et al.*, *Hepatology* 28: A620, 1998).

ADV is a substituted acyclic nucleoside phosphonate. This class of compounds also includes tenofovir disoproxil fumarate (also referred to as tenofovir DF, or tenofovir, or (TFV) or 9-R-(2-phosphonomethoxypropyl)adenine (PMPA) and is marketed as Viread by Gilead sciences).

IFV has antiviral activity against both HBV and HIV (Ying *et al.*, *J Viral Hepat.* 7(2): 161-165, 2000; Ying *et al.*, *J. Viral Hepat.* 7(1): 79-83, 2000; Suo *et al.*, *J Biol Chem.* 273(42): 27250-27258, 1998).

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FTC has activity against HBV and HIV (Frick *et al.*, *Antimicrob Agents Chemother* 37: 2285-2292, 1993).

10 Nucleoside or nucleotide analog therapy may be administered as monotherapy or combination therapy where two or more nucleoside or nucleotide analogs may be administered. The nucleoside or nucleotide analogs may also be administered in combination with other antiviral agents such as interferon or hepatitis B immunoglobulin (HBIG).

15 There is a need to monitor for the emergence of nucleoside/nucleotide-analog- or antibody-resistant strains of HBV and to develop diagnostic protocols to detect these resistant viruses and/or to use them to screen for and/or develop or design agents having properties making them useful as anti-viral agents. Defective forms of these resistant strains or antigenic components therefrom are also proposed to be useful in the development of
20 therapeutic vaccine compositions as are antibodies directed to viral surface components.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of the sequence identifiers is provided in Table 1. A sequence listing is provided after the claims.

Specific mutations in an amino acid sequence are represented herein as "Xaa₁nXaa₂" where Xaa₁ is the original amino acid residue before mutation, n is the residue number and Xaa₂ is the mutant amino acid. The abbreviation "Xaa" may be the three letter or single letter (i.e. "X") code. An "rt" before "Xaa₁nXaa₂" means "reverse transcriptase". An "s" means an envelope gene. The amino acid residues for HBV DNA polymerase are numbered with the residue methionine in the motif Tyr Met Asp Asp (YMDD) being residue number 204 (Stuyver *et al.*, *Hepatology* 33: 751-757, 2001). The amino acid residues for hepatitis B virus surface antigen are number according to Norder *et al.* (*J. Gen. Virol.* 74: 341-1348, 1993). Both single and three letter abbreviations are used to define amino acid residues and these are summarized in Table 2.

In accordance with the present invention, the selection of HBV variants is identified in patients (Patients A to L) with chronic HBV infection treated with ADV. Patient E is a nonresponder to ADV. Other variants of HBV are identified during a combination ADV and LMV treatment or a combination of TFV and LMV treatment (eg. Patent M) or a combination of TFV and FTC or treatment with TFV alone with mutations in the HBV DNA polymerase gene which reduce the sensitivity of HBV to nucleoside or nucleotide analogs. Consequently, HBV rt variants are contemplated which are resistant to, or which exhibit reduced sensitivity to, ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV;

LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof. Corresponding mutations in
5 the surface antigen also occur. The identification of these HBV variants is important for the development of assays to monitor ADV, LMV, FTC and/or TFV resistance and/or resistance to other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof and to screen for agents which are useful as alternative therapeutic agents.

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Reference herein to "anti-HBV agents" includes nucleoside and nucleotide analogs as well as immunological reagents (e.g. antibodies to HBV surface components) and chemical, proteinaceous and nucleic acid agents which inhibit or otherwise interfere with viral replication, maintenance, infection, assembly or release.

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The detection of such HBV variants is particularly important in the management of therapeutic protocols including the selection of appropriate agents for treating HBV infection. The method of this aspect of the present invention is predicated in part on monitoring the development in a subject of an increased HBV load in the presence of a
20 nucleoside or nucleotide analog or other anti-HBV agents or combinations thereof. The clinician is then able to modify an existing treatment protocol or select an appropriate treatment protocol accordingly.

Accordingly, one aspect of the present invention is directed to an isolated Hepatitis B virus
25 (HBV) variant wherein said variant comprises a nucleotide mutation in a gene encoding a DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to said DNA polymerase and wherein said variant exhibits decreased sensitivity to one or more nucleoside or nucleotide analogs selected from the list consisting of ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and
30 TFV; FTC and LMV; ADV and LMV and TFV; ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; ADV and FTC and LMV and TFV, and other

nucleoside or nucleotide analogs, and/or anti-HBV agents. The variant HBV comprises a mutation in an overlapping open reading frame in its genome in a region defined by one or more of domains F and G and domain A through to E of HBV DNA polymerase.

- 5 Another aspect of the present invention provides an isolated HBV variant comprising a nucleotide mutation in the S gene resulting in at least one amino acid addition, substitution and/or deletion to the surface antigen and which exhibits decreased sensitivity to one or more nucleoside or nucleotide analogs selected from the list consisting of ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC
10 and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and other nucleoside or nucleotide analogs and/or anti-HBV agents.

Useful mutants in the rt region include, in one embodiment, rtT38K, rtR55H and rtA181V;
15 in another embodiment include rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A, rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment include rtN238T; and yet another embodiment include rtI122V and rtA181T; in yet another embodiment include rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment include rtT128N and rtN236T, in yet
20 another embodiment include rtL180M, rtM204V, rtQ215S, in yet another embodiment include rtT128S, rtL180M, rtM204V and rtQ215S, in yet another embodiment include rtI80L, rtI204M, rtN238T, in yet another embodiment include rtN238T/A, in yet another embodiment include rtI187V, in yet another embodiment include rtN123N/I, rtS135Y, rtV214A/V and rtQ215Q/P/Stop/S, or a combination thereof or an equivalent mutation.

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Other HBV variants are also contemplated with mutations rtT38K (in the F domain of the DNA polymerase), rtR55H (located between the F and A domains), rtS/T78S, rtV80L (these are located within the A domain), rtN/S118N, rtI122V, rtN123N/I, rtS135Y, rtN/K139K, rtE142V (located between the A and B domains), rtA181V, rtA181T (these
30 are located in the B domain),; rtI187V (located between the B and C domains), rtA/V200V (Located in the C Domain), rtV214A, rtV214A/V and rtQ215Q/P/Stop/S,

rtQ/P/S/Stop215S, rtQ215S, rtE/K218E (located between the C and D domains), rtN236T, rtH237H/P rtN/H238H, rtN238T, rtN238T/A (these are located in the D domain), rtY245H (located between the D and E domains), and rtV253G (located in the E Domain) or a combination thereof or an equivalent mutation.

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Useful mutations in the S gene include, in one embodiment include sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another
 10 embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment
 15 include sV14A, in yet another embodiment include sL42R, sQ102Q/R, sT115T/S, sS207S/R, sL215R and sL216Stop, or a combination thereof or an equivalent mutation.

The present invention further contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV
 20 and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof by isolating DNA or corresponding mRNA from the HBV and screening for a mutation in the nucleotide
 25 sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G and domains A through to E or a region proximal thereto of the DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or
 30 ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV

and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The presence of such a mutation is an indication of the likelihood of resistance to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

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The present invention also provides a composition comprising a variant HBV resistant to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV, ADV and LMV and FTC and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof or an HBV surface antigen from the variant HBV or a recombinant or derivative form thereof or its chemical equivalent and one or more pharmaceutically acceptable carriers and/or diluents.

Yet another aspect of the present invention provides a use of the aforementioned composition or a variant HBV comprising a nucleotide mutation in a gene encoding a DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to the DNA polymerase and a decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof in the manufacture of a medicament for the treatment and/or prophylaxis of hepatitis B virus infection.

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The present invention also contemplates a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents or by isolating DNA or corresponding mRNA from the HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase wherein the presence of the

5 following mutations in the π region: in one embodiment, π T38K, π R55H and π A181V; in another embodiment include π S/T78S, π V80L, π N/S118N, π N/K139K, π E142V, π A/T181A, π I204M, π Q/P/S/Stop215S, π E/K218E, π N/H238H and π Y245H; in yet another embodiment include π N238T; and yet another embodiment include π I122V and π A181T; in yet another embodiment include π L180M, π A/V200V, π M204V, π V214A,

10 π H237H/P, π V253G, in yet another embodiment include π T128N and π N236T, in yet another embodiment include π L180M, π M204V π Q215S, in yet another embodiment includes π T128S π L180M, π M204V and π Q215S, in yet another embodiment includes π I80L, π I204M, π N238T, in yet another embodiment include π N238T/A, in yet another embodiment include π I187V in yet another embodiment include π N123N/I π S135Y,

15 π V214A/V and π Q215Q/P/Stop/S, or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV

20 and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

Still a further methodology comprises screening for a mutation in the nucleotide sequence encoding the envelope genes (s) wherein the presence of the following mutations in the s

25 gene: in one embodiment include sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F,

30 sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet

another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, in yet another embodiment include sL42R, sQ102Q/R, sT115T/S, sS207S/R, sL215R and sL216Stop or combinations thereof or an equivalent one or more other mutation is
5 indicative of a variant which exhibits a decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV, and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

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Preferably, the variants are in an isolated form such that they have undergone at least one purification step away from naturally occurring body fluid. Alternatively, the variants may be maintained in isolated body fluid or may be in DNA form. The present invention also contemplates infectious molecular clones comprising the genome or parts thereof from a
15 variant HBV. The detection of HBV or its components in cells, cell lysates, cultured supernatant fluid and bodily fluid may be by any convenient means including any nucleic acid-based detection means, for example, by nucleic acid hybridization techniques or *via* one or more polymerase chain reactions (PCRs). The term "bodily fluid" includes any fluid derived from the blood, lymph, tissue or organ systems including serum, whole blood,
20 biopsy and biopsy fluid, organ explants and organ suspension such as liver suspensions.

Another aspect of the present invention is directed to a variant HBV comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to a surface antigen from a reference or
25 wild type HBV and wherein an antibody generated to the reference or wild type surface antigen exhibits an altered immunological profile relative to the HBV variant. One altered profile includes a reduced capacity for neutralizing the HBV. More particularly, the surface antigen of the variant HBV exhibits an altered immunological profile compared to a pre-treatment HBV where the variant HBV is selected for by a nucleoside or nucleotide
30 analog or other anti-HBV agents of the HBV DNA polymerase. The variant HBV of this aspect of the invention may also comprise a nucleotide sequence comprising a single or

multiple nucleotide substitution, addition and/or deletion compared to a pre-treatment HBV.

The present invention further contemplates a method for detecting a variant HBV exhibiting an altered immunological profile said method comprising isolating an HBV from a subject exposed to a nucleoside or nucleotide analog or combination of analogs selected from the listed consisting of ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; ADV and FTC and LMV and TFV, and then contacting said HBV with a panel of one or more antibodies to a surface antigen and screening for any change in binding affinity or binding spectrum.

In a related invention, the present invention provides a method for detecting a variant HBV exhibiting an altered immunological profile said method comprising isolating a serum sample from a subject exposed to a nucleoside or nucleotide analog selected from the listed consisting of ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; ADV and FTC and LMV and TFV, and then contacting the serum with a panel of HBV surface antigens or antibody-binding fragments thereof and screening for any change in binding affinity or binding spectrum.

The present invention extends to an isolated HBsAg or a recombinant form thereof or derivative or chemical equivalent thereof corresponding to the variant HBV. Generally, the HBsAg or its recombinant or derivative form or its chemical equivalent comprises an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to an HBsAg from a reference HBV and wherein an antibody directed to a reference HBV exhibits an altered immunological profile to an HBV carrying said variant HBsAg. In one embodiment, the altered immunological profile comprises a reduction in the ability to neutralize the variant HBV.

Another aspect of the present invention contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV by generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in a plasmid vector and then transfecting said cells with said construct, contacting the cells, before, during and/or after transfection, with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agents; and the subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent. In a preferred embodiment, the plasmid vector is a baculovirus vector and the method comprises generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in or fused to an amount of a baculovirus genome effective to infect cells and then infecting said cells with said construct, contacting the cells, before, during and/or after infection, with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent and then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In connection with these methods, the plasmid vector may include genes encoding part or all of other viral vectors such as baculovirus vectors or adenovirus vectors (see Ren and Nassal, *J. Virol.* 75(3): 1104-1116, 2001).

In an alternative embodiment, the method comprises generating a continuous cell line comprising an infectious copy of the genome of the HBV in a replication competent effective amount such that said infectious HBV genome is stably integrated into said continuous cell line such as but not limited to the 2.2.15 or AD cell line, contacting the

cells with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to the agent and then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to
5 determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In an alternative embodiment, the present invention also contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV polymerase in an *in vitro*
10 polymerase assay. The HBV polymerase activity can be examined using established assays (Gaillard *et al.*, *Antimicrob Agents Chemother.* 46(4): 1005-1013, 2002; Xiong *et al.*, *Hepatology.* 28(6): 1669-73, 1998). The HBV polymerase may be a wild-type or reference HBV polymerase or mutant HBV polymerase.

15 The identification of viral variants enables the production of vaccines comprising particular recombinant viral components such as polymerases or envelope genes PreS1, PreS2, S encoding for L, M, S proteins as well as therapeutic vaccines comprising defective HBV variants. Rational drug design may also be employed to identify or generate therapeutic molecules capable of interacting with a polymerase or envelope genes
20 PreS1, PreS2, S encoding for L, M, S proteins or other component of the HBV. Such drugs may also have diagnostic potential. In addition, defective HBV variants may also be used as therapeutic compositions to generate an immune response against the same, similar or homologous viruses. Alternatively, antibodies generated to the HBV variants or surface components thereof may be used in passive immunization of subjects against infection by
25 HBV variants or similar or homologous viruses. Furthermore, agents such as nucleoside or nucleotide analogs, RNAi or siRNA molecules (both DNA-derived or synthetic), antisense or sense oligonucleotides, chemical or proteinaceous molecules having an ability to down-regulate the activity of a component of HBV and inhibit replication, maintenance, infection, assembly or release are contemplated by the present invention.

A summary of the abbreviations used throughout the subject specification are provided in Table 3

A summary of sequence identifiers used throughout the subject specification is provided in
5 Table 1.

TABLE 1
Summary of sequence identifiers

SEQUENCE ID NO:	DESCRIPTION
1	Formula I
2	Formula II
3	OS1 primer
4	TTA3 primer
5	JM primer
6	TTA4 primer
7	OS2 primer
8	sense primer
9	antisense primer
10	internal regions primer
11	internal regions primer
12	PC1 forward primer
13	PC2 reverse primer
14	HBV-specific molecular beacon primer

TABLE 2
Single and three letter amino acid abbreviations

Amino Acid	Three-letter Abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	The	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

TABLE 3
Abbreviations

ABBREVIATION	DESCRIPTION
3TC	(LMV); (-)-β-2'-deoxy-3'-thiacytidine
ADV	adefovir dipivoxil
DAPD	diaminopurine dioxalone
DXG	dioxolane guanine
ETV	entecavir
FAM	famciclovir
FCV	famciclovir
FTC	emtricitabine
HBIG	hepatitis B immunoglobulin
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
LMV	lamivudine
PMEA	9-[phosphonyl-methoxyethyl]-adenine; adefovir
PMPA	9-R-(2-phosphonomethoxypropyl)adenine
RNase	ribonuclease
rt ("rt" before "Xaa ₁ nXaa ₂ " means reverse transcriptase)	reverse transcriptase
s (as used in a mutation, e.g. sF134V)	envelope gene
TFV	tenofovir disoproxil fumarate
YMDD	Tyr Met Asp Asp-a motif in the polymerase protein; where the Met residue is designated residue number 204 of the reverse transcriptase

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing the partially double stranded DNA HBV genome showing the overlapping open reading frames encoding surface (S), core (C), polymerase (P) and X gene.

Figure 2 is a diagrammatic representation of the chemical structure of ADV.

Figure 3 is a diagrammatic representation of a computer system for determining the value of a variant HBV.

Figure 4 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient A during ADV treatment.

Figure 5 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient A during ADV therapy.

Figure 6 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient A during ADV therapy.

Figure 7 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient B during ADV and LMV treatment.

Figure 8 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient B during ADV and LMV therapy.

Figure 9 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient B during ADV and LMV therapy.

Figure 10 is a representation the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient C during ADV treatment.

Figure 11 is a representation the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient C during ADV therapy.

Figure 12 is a representation the deduced amino acid sequence of the envelope gene in samples from Patient C during ADV therapy.

Figure 13 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient D during ADV treatment.

Figure 14 is a representation the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient D during ADV therapy.

Figure 15 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient D during ADV therapy.

Figure 16 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient E during ADV treatment.

Figure 17 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient E during ADV therapy.

Figure 18 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient E during ADV therapy.

Figure 19 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient F during ADV treatment.

Figure 20 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient F during ADV therapy.

Figure 21 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient F during ADV therapy.

Figure 22 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient G during ADV treatment.

Figure 23 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient G during ADV therapy.

Figure 24 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient G during ADV therapy.

Figure 25 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient H during ADV treatment.

Figure 26 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient H during ADV therapy.

Figure 27 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient H during ADV therapy.

Figure 28 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient I during ADV treatment.

Figure 29 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient I during ADV therapy.

Figure 30 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient I during ADV therapy.

Figure 31 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient J during ADV treatment.

Figure 32 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient J during ADV therapy.

Figure 33 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient J during ADV therapy.

Figure 34 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient K during ADV treatment.

Figure 35 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient K during ADV therapy.

Figure 36 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient K during ADV therapy.

Figure 37 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient L during ADV treatment.

Figure 38 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient L during ADV therapy.

Figure 39 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient L during ADV therapy.

Figure 40 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient M during TFV treatment.

Figure 41 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient M during TFV therapy.

Figure 42 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient M during TFV therapy.

Figure 43 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in another sample from Patient M during TFV treatment.

Figure 44 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in another sample from Patient M during TFV therapy.

Figure 45 is a representation showing the deduced amino acid sequence of the envelope gene in another sample from Patient M during TFV therapy.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification and isolation of nucleoside or nucleotide analog-resistant variants of HBV following treatment of patients with either ADV or LMV or more particularly ADV and LMV or TFV and LMV, or optionally other nucleoside analogs or nucleotide analogs or other anti-HBV agents such as TFV or FTC. In particular, ADV or ADV and LMV treated patients gave rise to variants of HBV exhibiting decreased or reduced sensitivity to ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV. Reference herein to "decreased" or "reduced" in relation to sensitivity to ADV and/or LMV and/or FTC and/or TFV includes and encompasses a complete or substantial resistance to the nucleoside or nucleotide analog or other anti-HBV agents as well as partial resistance and includes a replication rate or replication efficiency which is more than a wild-type in the presence of a nucleoside or nucleotide analog or other anti-HBV agents. In one aspect, this is conveniently measured by an increase in viral load during treatment, or alternatively, there is no substantial decrease in HBV DNA viral load from pre-treatment HBV DNA levels during treatment (i.e., non-response to treatment).

Accordingly, one aspect of the present invention contemplates an isolated Hepatitis B virus (HBV) variant wherein said variant comprises a nucleotide mutation in a gene encoding a DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to said DNA polymerase and wherein said variant exhibits decreased sensitivity to one or more nucleoside or nucleotide analogs selected from the list consisting of ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; ADV and FTC and LMV and TFV, and other nucleoside or nucleotide analogs, and/or anti-HBV agents.

Another aspect of the present invention provides a method for determining the potential for an HBV to exhibit reduced sensitivity to a nucleoside or nucleotide analog selected from ADV, LMV, TFV and FTC or a combination thereof or optionally other nucleoside or nucleotide analogs, said method comprising isolating DNA or corresponding mRNA from
5 said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and A through E or a region proximal thereto of said DNA polymerase and associated with resistance or decreases sensitivity to one or more of ADV, LMV, TFV and/or FTC wherein the presence of such a mutation is an indication of the
10 likelihood of resistance to said one or more of ADV, LMV, TFV and/or FTC.

Before describing the present invention in detail, it is to be understood that unless otherwise indicated, the subject invention is not limited to specific formulations of components, manufacturing methods, dosage regimens, or the like, as such may vary. It is
15 also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for
20 example, reference to "a nucleoside or nucleotide analog" includes a single analog, as well as two or more analogs; reference to "an HBV variant" includes reference to two or more HBV variants; and so forth.

In describing and claiming the present invention, the following terminology is used in
25 accordance with the definitions set forth below.

The terms "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" are used interchangeably herein to refer to a chemical compound that induces a desired effect such as inhibit viral replication, infection,
30 maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. The terms also encompass pharmaceutically acceptable and pharmacologically active

ingredients of those active agents specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the terms "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" are used, then it is to be understood that this includes the active agent
5 *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc.

The present invention contemplates, therefore, compounds useful in inhibiting HBV replication, infection, maintenance, assembly and/or the function of an enzyme such as
10 HBV DNA polymerase. Reference to an "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" such as ADV, LMV, FTC and/or TFV includes combinations of two or more actives such as ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and
15 FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV. A "combination" also includes a two-part or more such as a multi-part anti-HBV therapeutic composition where the agents are provided separately and given or dispensed separately or admixed together prior to dispensation.

20 The terms "effective amount" and "therapeutically effective amount" of an agent as used herein mean a sufficient amount of the agent to provide the desired therapeutic or physiological effect of inhibiting HBV replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Furthermore, an "effective HBV-inhibiting amount" or "effective symptom-ameliorating amount" of an
25 agent is a sufficient amount of the agent to directly or indirectly inhibit replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount
30 required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible

to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

- 5 By "pharmaceutically acceptable" carrier, excipient or diluent is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e. the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emulsifying agents, pH
10 buffering agents, preservatives, and the like.

Similarly, a "pharmacologically acceptable" salt, ester, emide, prodrug or derivative of a compound as provided herein is a salt, ester, amide, prodrug or derivative that this not biologically or otherwise undesirable.

- 15 The terms "treating" and "treatment" as used herein refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, prevention of the occurrence of symptoms and/or their underlying cause, and improvement or remediation of damage in relation to HBV infection. Thus, for example, "treating" a
20 patient involves prevention of HBV infection as well as treatment of a clinically HBV symptomatic individual by inhibiting HBV replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Thus, for example, the present method of "treating" a patient with HBV infection or with a propensity for one to develop encompasses both prevention of HBV infection as well as treating HBV infection
25 or symptoms thereof. In any event, the present invention contemplates the treatment or prophylaxis of HBV infection.

- "Patient" as used herein refers to an animal, preferably a mammal and more preferably a primate including a lower primate and even more preferably, a human who can benefit
30 from the formulations and methods of the present invention. A patient regardless of whether a human or non-human animal may be referred to as an individual, subject,

animal, host or recipient. The compounds and methods of the present invention have applications in human medicine, veterinary medicine as well as in general, domestic or wild animal husbandry. For convenience, an "animal" includes an avian species such as a poultry bird (including ducks, chicken, turkeys and geese), an aviary bird or game bird.

5 The condition in a non-human animal may not be a naturally occurring HBV infection but HBV-like infection may be induced.

As indicated above, the preferred animals are humans, non-human primates such as marmosets, baboons, orangatangs, lower primates such as tupia, livestock animals,

10 laboratory test animals, companion animals or captive wild animals. A human is the most preferred target. However, non-human animal models may be used.

Examples of laboratory test animals include mice, rats, rabbits, guinea pigs and hamsters. Rabbits and rodent animals, such as rats and mice, provide a convenient test system or

15 animal model as do primates and lower primates. Livestock animals include sheep, cows, pigs, goats, horses and donkeys. Non-mammalian animals such as avian species, zebrafish, amphibians (including cane toads) and *Drosophila* species such as *Drosophila melanogaster* are also contemplated. Instead of a live animal model, a test system may also comprise a tissue culture system.

20

An "anti-HBV agent" includes a nucleoside or nucleotide analog, protein, chemical compound, RNA or DNA or RNAi or siRNA oligonucleotide (either DNA-derived or synthetic).

25 Preferably, the decreased sensitivity is in respect of ADV. Alternatively, the decreased sensitivity is in respect of LMV. Alternatively, the decreased sensitivity is in respect of TFV. Alternatively, the decreased sensitivity is in respect of FTC. Alternatively, the decreased sensitivity is in respect of ADV and LMV. Alternatively, the decreased sensitivity is in respect of ADV and TFV. Alternatively, the decreased sensitivity is in

30 respect of LMV and TFV. Alternatively, the decreased sensitivity is in respect of ADV and FTC. Alternatively, the decreased sensitivity is in respect to FTC and TFV. Alternatively,

the decreased sensitivity is in respect of FTC and LMV. Alternatively, the decreased sensitivity is in respect of ADV and LMV and TFV. Alternatively, the decreased sensitivity is in respect to ADV and TFV and FTC. Alternatively, the decreased sensitivity is in respect to LMV and TFV and FTC. Alternatively, the decrease sensitivity is in respect
5 of ADV and LMV and FTC. Alternatively, the decreased sensitivity is in respect of ADV and FTC and TFV and LMV.

Reference herein to "anti-HBV agents" includes nucleoside and nucleotide analogs as well as immunological reagents (e.g. antibodies to HBV surface components) and chemical,
10 proteinaceous and nucleic acid agents which inhibit or otherwise interfere with viral replication, maintenance, infection, assembly or release. Reference herein to "nucleic acid" includes reference to a sense or antisense molecule, RNA or DNA, oligonucleotides and RNAi and siRNA molecules and complexes containing same.

15 In addition to a mutation in the gene encoding DNA polymerase, due to the overlapping nature of the HBV genome (Figure 1), a corresponding mutation may also occur in the gene encoding the S gene encoding the surface antigen (HBsAg) resulting in reduced interactivity of immunological reagents such as antibodies and immune cells to HBsAg. The reduction in the interactivity of immunological reagents to a viral surface component
20 generally includes the absence of immunological memory to recognize or substantially recognize the viral surface component. The present invention extends, therefore, to an HBV variant exhibiting decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and
25 LMV and FTC; and/or ADV and FTC and LMV and TFV or a reduced interactivity of an immunological reagent to HBsAg wherein the variant is selected for following ADV and/or LMV combination or sequential treatment. The term "sequential" in this respect means ADV followed by LMV and/or TFV, and /or FTC, LMV followed by ADV and/or TFV, and /or FTC, or multiple sequential administrations of each of ADV, LMV and/or
30 TFV, and /or FTC.

A viral variant may, therefore, carry A mutation only in the DNA polymerase gene or both in the DNA polymerase gene and the S gene. The term "mutation" is to be read in its broadest context and includes multiple mutations.

- 5 The present invention extends to a mutation and any domain of the HBV DNA polymerase and in particular regions F and G, and domains A through to E provided said mutation leads to decreased sensitivity to ADV and/or LMV and/or TFV or combinations thereof.

10 In this specification, reference is particularly made to the conserved regions of the DNA polymerase as defined by domains A to E. Regions A to E are defined by the amino acid sequence set forth in Formula II in Australian Patent No. 734831.

Preferably, the mutation results in an altered amino acid sequence in any one or more of domains F and G, and domains A through to E or regions proximal thereto of the HBV
15 DNA polymerase.

Another aspect of the present invention provides an HBV variant comprising a mutation in an overlapping open reading frame in its genome wherein said mutation is in a region defined by one or more of domains F and G, and domains A through to E of HBV DNA
20 polymerase and wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents.

25

Another preferred aspect of the present invention contemplates an HBV variant comprising a mutation in the nucleotide sequence encoding HBsAg resulting in an amino acid addition, substitution and/or deletion in said HBsAg wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV;
30 LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or

ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

More particularly, the present invention provides a variant HBV comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to a surface antigen from a reference or wild-type HBV and wherein an antibody generated to the reference or wild-type surface antigen exhibits reduced capacity for neutralizing said HBV variant, said variant selected by exposure of a subject to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; 5 LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

15 The term "combination therapy" means that both combinations of ADV, LMV, FTC and/or TFV are co-administered in the same composition or simultaneously in separate compositions. The term "sequential therapy" means that the two agents are administered within seconds, minutes, hours, days or weeks of each other and in either order. Sequential therapy also encompasses completing a therapeutic course with one or other of ADV, 20 LMV, FTC or TFV and then completing a second or third or subsequent therapeutic courses with the other of ADV, LMV, FTC or TFV.

Accordingly, another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to LMV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV and LMV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and
5 wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV and TFB therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

10 A further aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure
15 of a subject to LMV and TFB therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution,
20 addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

25

Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile
30 compared to the pretreatment HBV where the said variant HBV is selected for by exposure

of a subject to TFV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

5 Still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to FTC and LMV therapy or therapy by one or more other nucleoside or
10 nucleotide analogs or other anti-HBV agents.

Even yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and
15 wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

20 Even still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure
25 of a subject to ADV, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

A further aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid
30 substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile

compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

- 5 Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject
10 to FTC, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

- Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid
15 substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, FTC and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

20

- Still yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile
25 compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV, FTC and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

- Preferably, the variants are in isolated form such that they have undergone at least one
30 purification step away from naturally occurring body fluid. Alternatively, the variants may be maintained in isolated body fluid or may be in DNA form. The present invention also

contemplates infectious molecular clones comprising the genome or parts thereof from a variant HBV. Furthermore, the present invention provides isolated components from the variant HBVs such as but not limited to an isolated HBsAg. Accordingly, the present invention provides an isolated HBsAg or a recombinant form thereof or derivative or
5 chemical equivalent thereof, said HBsAg being from a variant HBV selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or optionally one or more nucleoside or nucleotide analogs or other anti-HBV agents.

More particularly, yet another aspect of the present invention is directed to an isolated
10 variant HBsAg or a recombinant or derivative form thereof or a chemical equivalent thereof wherein said HBsAg or its recombinant or derivative form or its chemical equivalent exhibits an altered immunological profile compared to an HBsAg from a reference HBV, said HBsAg being from a variant HBV selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or optionally one or more nucleoside or
15 nucleotide analogs or other anti-HBV agents.

Even more particularly, the present invention provides an isolated variant HBsAg or a recombinant or derivative form thereof or a chemical equivalent thereof wherein said HBsAg or its recombinant or derivative form or its chemical equivalent comprises an
20 amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to an HBsAg from a reference HBV and wherein a neutralizing antibody directed to a reference HBV exhibits no or reduced neutralising activity to an HBV carrying said variant HBsAg, said HBsAg being from a variant HBV selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or
25 optionally one or more nucleoside or nucleotide analogs or other anti-HBV agents.

Preferred mutations in the HBV DNA polymerase include variants selected from patients with HBV recurrence following ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV
30 and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV treatment. Nucleoside or nucleotide analogs or other

anti-HBV agents may be indicated during , after or prior to a transplantation procedure (e.g. bone marrow transplantation (BMT) or OLT) or following treatment of patients diagnosed with hepatitis. Following selection of variants, viral loads are obtainable at levels similar to pre-treatment levels or increase while on therapy.

5

Useful mutants in the rt region include, in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment include rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A, rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment include rtN238T; and yet another embodiment include rtI122V and
 10 rtA181T; in yet another embodiment include rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment include rtT128N and rtN236T, in yet another embodiment include rtL180M, rtM204V and rtQ215S, in yet another embodiment include rtT128S, rtL180M, rtM204V and rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment include rtN238T/A, in yet another
 15 embodiment include rtI187V, in yet another embodiment include rtN123N/I, rtS135Y, rtV214A/V and rtQ215Q/P/Stop/S, or a combination thereof or an equivalent mutation

Such HBV variants are proposed to exhibit a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV;
 20 FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. It should be noted that the nomenclature system for amino acid positions is based on the methionine residues in the YMDD motif being designated codon rtM204. This
 25 numbering system is different to that in Australian Patent No. 734831 where the methionine residue in the YMDD motif within the polymerase gene is designated codon 550. In this regard, rtL180M and rtM204V correspond to L526M and M550V, respectively, in Australian Patent No. 734831. Corresponding mutations may also occur in envelope genes such as in one or more of PreS1, PreS2 and S.

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Another potential mode of action of ADV and other acyclic nucleoside phosphonates is that of immune stimulation (Calio *et al.*, *Antiviral Res.* 23: 77-89, 1994). A number of mutations resulted in changes in the envelope gene detected in HBV variants which may be associated with immune escape. These changes include in one embodiment include in one embodiment include sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, in yet another embodiment include sL42R, sQ102Q/R, sT115T/S, sS207S/R, sL215R and sL216Stop, or a combination thereof or an equivalent mutation.

The identification of the variants of the present invention permits the generation of a range of assays to detect such variants. The detection of such variants may be important in identifying resistant variants to determine the appropriate form of chemotherapy and/or to monitor vaccination protocols, or develop new or modified vaccine preparations.

Still another aspect of the present invention contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G, and A domains through to E or a region proximal thereto of said DNA polymerase and associated

with resistance or decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or
 5 nucleotide analogs or other anti-HBV agents wherein the presence of such a mutation is an indication of the likelihood of resistance to said ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or
 10 nucleotide analogs or other anti-HBV agents.

Accordingly, another aspect of the present invention provides a method for determining the potential for an HBV to exhibit reduced sensitivity to a nucleoside or nucleotide analog selected from ADV, LMV, TFV and FTC or optionally other nucleoside or nucleotide
 15 analogs, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and A through E or a region proximal thereto of said DNA polymerase and associated with resistance or decreases sensitivity to one or more of ADV, LMV, TFV
 20 and/or FTC wherein the presence of such a mutation is an indication of the likelihood of resistance to said one or more of ADV, LMV, TFV and/or FTC.

Preferably, the assay detects one or more of the following mutations: in one embodiment, rT38K, rR55H and rA181V; in another embodiment include rS/T78S, rV80L,
 25 rN/S118N, rN/K139K, rE142V, rA/T181A rI204M, rQ/P/S/Stop215S, rE/K218E, rN/H238H and rY245H; in yet another embodiment include rN238T; and yet another embodiment include rI122V and rA181T; in yet another embodiment include rL180M, rA/V200V, rM204V, rV214A, rH237H/P, rV253G, in yet another embodiment include rT128N and rN236T, in yet another embodiment include rL180M, rM204V rQ215S, in
 30 yet another embodiment include rT128S rL180M, rM204V and rQ215S, in yet another embodiment include rI80L, rI204M, rN238T, in yet another embodiment include

- rtN238T/A, in yet another embodiment include rtI187V, in yet another embodiment include rtN123N/I rtS135Y, rtV214A/V and rtQ215Q/P/Stop/S, or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.
- 10 Accordingly, another aspect of the present invention produces a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase and/or a corresponding region of the S gene, wherein the presence of a
- 15 mutation selected from, in one embodiment include sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sL/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2
- 20 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53I, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, in yet another embodiment include sL42R, sQ102Q/R, sT115T/S,
- 25 sS207S/R, sL215R and sL216Stop and in yet another embodiment include sT47A and sW172stop, in even still another embodiment, rtT38K, rtR55H and rtA181V; in another embodiment include rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A, rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment include rtN238T; and yet another embodiment include rtI122V and rtA181T;
- 30 in yet another embodiment include rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment include rtT128N and rtN236T, in yet another

embodiment include rL180M, rM204V rQ215S, in yet another embodiment include rT128S rL180M, rM204V and rQ215S, in yet another embodiment includes rI180L, rI204M, rN238T, in yet another embodiment include rN238T/A, in yet another embodiment include rI187V, in yet another embodiment include rN123N/I rS135Y, rV214A/V and rQ215Q/P/Stop/S, or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

A further aspect of the present invention produces a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase and/or a corresponding region of the S gene, wherein the presence of a mutation selected from, in one embodiment, sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, in yet another embodiment include sL42R, sQ102Q/R, sT115T/S, sS207S/R, sL215R and sL216Stop and in yet another embodiment include sT47A and sW172stop, in even still another embodiment, rT38K, rR55H and rA181V; in another embodiment include rT5/T78S, rV80L, rN/S118N, rN/K139K, rE142V, rA/T181A rI204M, rQ/P/S/Stop215S, rE/K218E, rN/H238H and rY245H; in yet another

embodiment include rtN238T; and yet another embodiment include rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment include rtT128N and rtN236T, in yet another embodiment include rtL180M, rtM204V rtQ215S, in yet another embodiment
 5 include rtT128S rtL180M, rtM204V and rtQ215S, in yet another embodiment include rtI80L, rtI204M, rtN238T, in yet another embodiment include rtN238T/A, in yet another embodiment include rtI187V, in yet another embodiment include rtN123N/I rtS135Y, rtV214A/V and rtQ215Q/P/Stop/S, combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV,
 10 LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

15
 The detection of HBV or its components in cells, cell lysates, cultured supernatant fluid and bodily fluid may be by any convenient means including any nucleic acid-based detection means, for example, by nucleic acid hybridization techniques or *via* one or more polymerase chain reactions (PCRs). The term "bodily fluid" includes any fluid derived
 20 from the blood, lymph, tissue or organ systems including serum, whole blood, biopsy and biopsy fluid, organ explants and organ suspension such as liver suspensions. The invention further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single-strand chain polymorphism (SSCP), amplification
 25 and mismatch detection (AMD), interspersed repetitive sequence polymerase chain reaction (IRS-PCR), inverse polymerase chain reaction (iPCR) and reverse transcription polymerase chain reaction (RT-PCR), amongst others. Other forms of detection include Northern blots, Southern blots, PCR sequencing, antibody procedures such as ELISA, Western blot and immunohistochemistry. A particularly useful assay includes the reagents
 30 and components required for immobilized oligonucleotide- or oligopeptide-mediated detection systems.

One particularly useful nucleic acid detection system is the reverse hybridization technique. In this technique, DNA from an HBV sample is amplified using a biotin or other ligand-labeled primer to generate a labeled amplicon. Oligonucleotides
5 immobilized to a solid support such as a nitrocellulose film are then used to capture amplified DNA by hybridization. Specific nucleic acid fragments are identified *via* biotin or the ligand. Generally, the labeled primer is specific for a particular nucleotide variation to be detected. Amplification occurs only if the variation to be detected is present. There are many forms of the reverse hybridization assay and all are encompassed by the present
10 invention.

Another aspect contemplated by the present invention provides a method for detecting a variant HBV exhibiting an altered immunological profile said method comprising isolating an HBV from a subject exposed to a nucleoside or nucleotide analog selected from the
15 listed consisting of ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; ADV and FTC and LMV and TFV, and then contacting said HBV with a panel of one or more antibodies to a surface antigen and screening for any change in binding affinity or binding spectrum.
20

In a related embodiment, the present invention contemplates a method for detecting a variant HBV exhibiting an altered immunological profile said method comprising isolating a serum sample from a subject exposed to a nucleoside or nucleotide analog selected from the listed consisting of ADV, LMV, TFV or FTC; ADV and LMV; ADV and TFV; LMV
25 and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; ADV and FTC and LMV and TFV, and then contacting the serum with a panel of HBV surface antigens or antibody-binding fragments thereof and screening for any change in binding affinity or binding spectrum.
30

Detecting HBV replication in cell culture is particularly useful.

This and other aspects of the present invention is particularly amenable to microarray analysis such as to identify oligonucleotides including sense and antisense molecules, RNAi or siRNA molecules or DNA or RNA-binding molecules which down-regulate
5 genomic sequences or transcripts of HBV. Microarray analysis may also be used to identify particular mutations in the HBV genome such as within the HBV DNA polymerase-coding region or the HBsAg-coding region.

Another aspect of the present invention contemplates a method for detecting an agent
10 which exhibits inhibitory activity to an HBV by:

generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in a plasmid vector and then transfecting said cells with said construct;

15

contacting the cells, before, during and/or after transfection, with the agent to be tested;

culturing the cells for a time and under conditions sufficient for the HBV to
20 replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agents; and

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated,
25 expressed genetic material and/or assembled and/or been released in the presence of the agent.

In a preferred embodiment, the plasmid vector may include genes encoding part or all of other viral vectors such as baculovirus or adenovirus (Ren and Nassal, 2001, *supra*) and
30 the method comprises:

generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in or fused to an amount of a baculovirus genome or adenovirus genome effective to infect cells and then infecting said cells with said construct;

5

contacting the cells, before, during and/or after infection, with the agent to be tested;

10 culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated,
15 expressed genetic material and/or assembled and/or been released in the presence of the agent.

In an alternative embodiment, the method comprises:

20 generating a continuous cell line comprising an infectious copy of the genome of the HBV in a replication competent effective amount such that said infectious HBV genome is stably integrated into said continuous cell-line such as but not limited to 2.2.15 or AD;

25 contacting the cells with the agent to be tested;

culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to the agent; and

30

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

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The above-mentioned methods are particularly useful in identifying or developing agents against HBV variants such as those carrying mutations, in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment include rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and
 10 rtY245H; in yet another embodiment include rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment include rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment include rtT128N and rtN236T, in yet another embodiment include rtL180M, rtM204V rtQ215S, in yet another embodiment include rtT128S rtL180M, rtM204V and rtQ215S, in yet another embodiment
 15 include rtI80L, rtI204M, rtN238T, in yet another embodiment include rtN238T/A, in yet another embodiment includes rtI187V, in yet another embodiment includes rtN123N/I rtS135Y, rtV214A/V and rtQ215Q/P/Stop/S, or a combination thereof or an equivalent mutation; in a further embodiment, sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N,
 20 sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sL208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet
 25 another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, in yet another embodiment include sL42R, sQ102Q/R, sT115T/S, sS207S/R, sL215R and sL216Stop, or a combination thereof or an equivalent mutation.

30 Accordingly, another aspect of the present invention contemplates a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or

nucleotide analog or other potential anti-HBV agent, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence of the envelope genes or DNA polymerase gene selected from, in one embodiment, in one embodiment, rT38K, rR55H and rA181V; in another embodiment includes rS/T78S, rV80L, rN/S118N, rN/K139K, rE142V, rA/T181A rI204M; rQ/P/S/Stop215S, rE/K218E, rN/H238H and rY245H; in yet another embodiment include rN238T; and yet another embodiment include rI122V and rA181T; in yet another embodiment include rL180M, rA/V200V, rM204V, rV214A, rH237H/P, rV253G, in yet another embodiment include rT128N and rN236T, in yet another embodiment include rL180M, rM204V rQ215S, in yet another embodiment include rT128S rL180M, rM204V and rQ215S, in yet another embodiment include rI80L, rI204M, rN238T, in yet another embodiment include rN238T/A, in yet another embodiment include rI187V, in yet another embodiment include rN123N/I rS135Y, rV214A/V and rQ215Q/P/Stop/S, or a combination thereof or an equivalent mutation; in a further embodiment, sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, in yet another embodiment include sL42R, sQ102Q/R, sT115T/S, sS207S/R, sL215R and sL216Stop, or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The detection of amino acid variants of DNA polymerase is conveniently accomplished by a range of amino acid detection techniques. Where an HBV variant comprises an amino acid change, then such an isolate is considered a putative HBV variant having an altered DNA polymerase activity.

The present invention further contemplates agents which inhibit ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV resistant HBV variants. Such agents are particularly useful if long term treatment by ADV, LMV, FTC and/or TFV and/or optionally other nucleoside or nucleotide analogs such as TFV is contemplated by the clinician. The agents may be DNA or RNA or proteinaceous or non-proteinaceous chemical molecules. Natural product screening such as from plants, coral and microorganisms is also contemplated as a useful potential source of masking agents as is the screening of combinatorial or chemical libraries. The agents may be in isolated form or in the form of a pharmaceutical composition or formulation and may be administered in place of or sequentially or simultaneously with a nucleoside or nucleotide analog. Furthermore, rationale drug design is contemplated including solving the crystal or NMR structure of, for example, HBV DNA polymerase and designing agents which can bind to the enzyme's active site. This approach may also be adapted to other HBV components.

Accordingly, another aspect of the present invention contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV which exhibits resistance or decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in a plasmid vector and then transfecting said cells with said construct;

5 contacting said cells, before, during and/or after transfection, with the agent to be tested;

 culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like
10 particles if resistant to said agent; and

 subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

15

Still another aspect of the present invention provides a method for detecting an agent which exhibits inhibitory activity to an HBV which exhibits resistance or decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or
20 ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, , said method comprising:

 generating a genetic construct comprising a replication competent-effective
25 amount of the genome from said HBV contained in or fused to an amount of a baculovirus genome effective to infect cells and then infecting said cells with said construct;

 contacting said cells, before, during and/or after infection, with the agent to be tested;

30

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

- 5 subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

Preferably, the HBV genome is stably integrated into the cells' genome.

10

Particularly useful cells are 2.2.15 cells (Price *et al.*, *Proc. Natl. Acad. Sci. USA* 86(21): 8541-8544, 1989 or AD cells (also known as HepAD32 cells or HepAD79 cells [Ying *et al.*, *Viral Hepat.* 7(2): 161-165, 2000.

- 15 Whilst the baculovirus vector is a particularly useful in the practice of the present invention, the subject invention extends to a range of other vectors such as but not limited to adenoviral vectors.

- 20 The present invention further extends to cell lines (e.g. 2.2.15 or AD cells) carrying genetic constructs comprising all or a portion of an HBV genome or a gene or part of a gene therefrom.

- 25 The present invention also provides for the use of the subject HBV variants to screen for anti-viral agents. These anti-viral agents inhibit the virus. The term "inhibit" includes antagonizing or otherwise preventing infection, replication, assembly and/or release or any intermediate step. Preferred anti-viral agents include nucleoside or nucleotide analogs or anti-HBV agents, however, the present invention extends to non-nucleoside molecules.

- 30 In addition, rational drug design is also contemplated to identify or generate chemical molecules which either mimic a nucleoside or which interact with a particular nucleotide sequence or a particular nucleotide. Combinatorial chemistry and two hybrid screening are

some of a number of techniques which can be employed to identify potential therapeutic or diagnostic agents.

In one example, the crystal structure or the NMR structure of polymerase or the surface antigen is used to rationally design small chemical molecules likely to interact with key regions of the molecule required for function and/or antigenicity. Such agents may be useful as inhibitors of polymerase activity and/or may alter an epitope on the surface antigen.

10 Several models of the HBV polymerase have been prepared due to the similarity with reverse transcriptase from HIV (Das *et al.*, *J. Virol.* 75(10): 4771-4779, 2001; Bartholomeusz *et al.*, *Intervirology* 40(5-6): 337-342 1997; Allen *et al.*, *Hepatology* 27(6): 1670-1677, 1998). The models of the HBV polymerase can be used for the rational drug design of new agents effective against HBV encoding the resistant mutations as well as
15 wild type virus. The rational drug that is designed may be based on a modification of an existing antiviral agent such as the agent used in the selection of the HBV encoding the mutations associated with resistance. Viruses or clones expressing HBV genomic material encoding the mutations may also be used to screen for new antiviral agents.

20 In an alternative embodiment, the present invention also contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV polymerase in an *in vitro* polymerase assay. The HBV polymerase activity can be examined using established assays (Gaillard *et al.*, *Antimicrob Agents Chemother.* 46(4): 1005-1013, 2002; Xiong *et al.*, *Hepatology* 28(6): 1669-1673, 1998).

25

As indicated above, microarray technology is also a useful means of identifying agents which are capable of interacting with defined HBV internal or external components. For example, arrays of HBV DNA polymerase or peptide fragments thereof carrying different amino acid variants may be used to screen for agents which are capable of binding or
30 otherwise interacting with these molecules. This is a convenient way of determining the differential binding patterns of agents between HBV variants. Arrays of antibodies may

also be used to screen for altered HBsAg molecules. Microarrays are also useful in proteomic analysis to identify molecules such as antibodies, interferons or cytokines which have an ability to interact with an HBV component. Microarrays of DNA and RNA molecules may also be employed to identify sense and antisense molecules for genetic regions on the HBV genome or transcripts thereof.

The above methods are particularly useful in identifying an inhibitor of an HBV resistant to or exhibiting reduced sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. The present invention extends, therefore, to compositions of the inhibitors. The inhibitors may also be in the form of antibodies or genetic molecules such as ribozymes, antisense molecules and/or sense molecules for co-suppression or the induction of RNAi or may be other nucleoside or nucleotide analogs or other anti-HBV agents or derivatives of known analogs. Reference to RNAi includes reference to short, interfering RNAs (siRNA) and all RNAi-type molecules may be DNA-derived or synthetic.

The term "composition" includes a "pharmaceutical composition" or a formulation.

The inhibitor is referred to below as an "active ingredient" or "active compound" and may be selected from the list of inhibitors given above.

The composition may include an antigenic component of the HBV, a defective HBV variant or an agent identified through natural product screening or rational drug design (including combinatorial chemistry).

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active

substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

5

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of encoding an aspartyl protease inhibitor. The vector may, for example, be a viral vector.

- 10 Pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dilution medium comprising, for example, water, ethanol,
- 15 polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of surfactants. The preventions of the action of microorganisms can be brought about by various anti-bacterial and anti-fungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it
- 20 will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.
- 25 Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with the active ingredient and optionally other active ingredients as required, followed by filtered sterilization or other appropriate means of sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, suitable methods of preparation include vacuum drying and the freeze-drying
- 30 technique which yield a powder of active ingredient plus any additionally desired ingredient.

When the active ingredient is suitably protected, it may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets. For oral
5 therapeutic administration, the active ingredient may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight
10 of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μ g and 200 mg of active compound. Alternative dosage amounts include from about 1 μ g to about 1000 mg and from about 10 μ g to about 500 mg. These
15 dosages may be per individual or per kg body weight. Administration may be per hour, day, week, month or year.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as
20 dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be
25 present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavouring. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-
30 toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

As stated above, the present invention further extends to an isolated HBsAg from the HBV variants herein described. More particularly, the present invention provides an HBsAg or a recombinant form thereof or derivative or chemical equivalent thereof. The isolated surface component and, more particularly, isolated surface antigen or its recombinant, derivative or chemical equivalents are useful in the development of biological compositions such as vaccine formulations.

Yet another aspect of the present invention provides a composition comprising a variant HBV resistant to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or an HBV surface antigen from said variant HBV or a recombinant or derivative form thereof or its chemical equivalent and one or more pharmaceutically acceptable carriers and/or diluents. Such a composition may be regarded as a therapeutic composition and is useful in generating an immune response including a humoral response. Generally, the HBV variants are "defective" and in themselves are unable to cause a sustained infection in a subject.

20

As indicated above, antibodies may be generated to the mutant HBV agents and used for passive or direct vaccination against infection by these viruses. The antibodies may be generated in humans or non-human animals. In the case of the latter, the non-human antibodies may need to be deimmunized or more specifically humanized prior to use. Deimmunized may include, for example, grafting complementarity determining regions (CDRs) from the variable region of a murine or non-human animal anti-HBV antibody onto a human consensus fragment antibody binding (Fab) polypeptide. Alternatively, amino acids defining epitopes in the variable region of the antibody may be mutated so that the epitopes are no longer recognized by the human MHC II complex.

30

Insofar as ribozyme, antisense or co-suppression (RNAi) or siRNA or complexes thereof repression is concerned, this is conveniently aimed at post-transcription gene silencing. DNA or RNA may be administered or a complex comprising RNAi or a chemical analog thereof specific for HBV mRNA may be employed.

5

All such molecules may be incorporated into pharmaceutical compositions.

In another embodiment, the present invention provides a biological composition comprising a variant HBV or an HBsAg or L, M or S proteins from said variant HBV or a
10 recombinant or derivative form thereof or its chemical equivalent.

Generally, if an HBV is used, it is first attenuated. The biological composition according to this aspect of the present invention generally further comprises one or more pharmaceutically acceptable carriers and/or diluents.

15

The biological composition may comprise HBsAg or like molecule from one HBV variant or the composition may be a cocktail of HbsAgS or L, M or S proteins or like molecules from a range of ADV- and/or LMV- and/or, FTC- and/or TFV-resistant HBV variants. Similar inclusions apply where the composition comprises an HBV.

20

The present invention is further directed to the use of defective HBV variants in the manufacture of therapeutic vaccines to vaccinate individuals against infection by HBV strains having a particular nucleotide sequence or encoding a particular polymerase or surface antigen or L, M or S proteins.

25

Examples of suitable vaccine candidates are defective forms of HBV variants comprising a mutation selected from, in one embodiment, in one embodiment, rT38K, rR55H and rA181V; in another embodiment include rS/T78S, rV80L, rN/S118N, rN/K139K, rE142V, rA/T181A rI204M, rQ/P/S/Stop215S, rE/K218E, rN/H238H and rY245H; in
30 yet another embodiment include rN238T; and yet another embodiment include rI122V and rA181T; in yet another embodiment include rL180M, rA/V200V, rM204V,

rV214A, rH237H/P, rV253G, in yet another embodiment include rT128N and rN236T,
 in yet another embodiment include rL180M, rM204V rQ215S, in yet another embodiment
 include rT128S rL180M, rM204V and rQ215S, in yet another embodiment include
 rI180L, rI204M, rN238T, in yet another embodiment include rN238T/A, in yet another
 5 embodiment include rI187V, in yet another embodiment include rN123N/I rS135Y,
 rV214A/V and rQ215Q/P/Stop/S, or a combination thereof or an equivalent mutation; in
 a further embodiment, sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another
 embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y,
 sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A,
 10 sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and
 sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F,
 sI195M, and in yet another embodiment include sS53L, and in yet another embodiment
 include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet
 another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment
 15 include sL95W and sL196W, and in yet another embodiment include sV14A, in yet
 another embodiment include sL42R, sQ102Q/R, sT115T/S, sS207S/R, sL215R and
 sL216Stop, or a combination thereof or an equivalent mutation.

In one embodiment, for example, an HBV variant may be identified having a particular
 20 mutation in its polymerase conferring resistance or decreased sensitivity to a nucleoside or
 nucleotide analog. This variant may then be mutated to render it defective, i.e. attenuated
 or unable to cause infection. Such a defective, nucleoside or nucleotide analog-resistant
 virus may then be used as a therapeutic vaccine against virulent viruses having the same
 mutation in its polymerase.

25

The subject invention extends to kits for assays for variant HBV resistant to ADV, LMV,
 TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC
 and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV
 and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV.
 30 Such kits may, for example, contain the reagents from PCR or other nucleic acid
 hybridization technology or reagents for immunologically based detection techniques. A

particularly useful assay includes the reagents and components required for immobilized oligonucleotide- or oligopeptide-mediated detection systems.

Still another aspect of the present invention contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G, and domains A through to E or a region proximal thereto of said DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV, wherein the presence of such a mutation is an indication of the likelihood of resistance to said ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV.

An assessment of a potential viral variant is important for selection of an appropriate therapeutic protocol. Such an assessment is suitably facilitated with the assistance of a computer programmed with software, which *inter alia* adds input codes for at least two features associated with the viral variants to provide a value corresponding to the resistance or sensitivity of a viral variant to a particular chemical compound or immunological agent. The value can be selected from (a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent; (b) an altered DNA polymerase from wild-type HBV; (c) an altered surface antigen from wild-type HBV; or (d) morbidity or recovery potential of a patient. Thus, in accordance with the

present invention, the values for such features are stored in a machine-readable storage medium, which is capable of processing the data to provide a value for a particular viral variant or a biological specimen comprising same.

5 Thus, in another aspect, the invention contemplates a computer program product for assessing the likely usefulness of a viral variant or biological sample comprising same for determining an appropriate therapeutic protocol in a subject (Figure 3), said product comprising:

10 (1) code that receives as input code for at least two features associated with said viral agents or biological sample comprising same, wherein said features are selected from:

- 15 (a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent;
- (b) an altered DNA polymerase from wild-type HBV;
- (c) an altered surface antigen from wild-type HBV; or
- (d) morbidity or recovery potential of a patient;

20 (2) code that adds said input code to provide a sum corresponding to a value for said viral variants or biological samples; and

(3) a computer readable medium that stores the codes.

25 In a related aspect, the invention extends to a computer for assessing the likely usefulness of a viral variant or biological sample comprising same in a subject, wherein said computer comprises:

30 (1) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said machine-readable data comprise input codes for at least two features associated

with said viral variant or biological sample; wherein said features are selected from:-

- 5 (a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent;
 - (b) an altered DNA polymerase from wild-type HBV;
 - (c) an altered surface antigen from wild-type HBV; or
 - (d) morbidity or recovery potential of a patient;
- 10 (2) a working memory for storing instructions for processing said machine-readable data;
- (3) a central-processing unit coupled to said working memory and to said machine-readable data storage medium, for processing said machine
- 15 readable data to provide a sum of said input code corresponding to a value for said compound(s); and
- (4) an output hardware coupled to said central processing unit, for receiving said value.

20

Any general or special purpose computer system is contemplated by the present invention and includes a processor in electrical communication with both a memory and at least one input/output device, such as a terminal. Figure 3 shows a generally suitable computer system. Such a system may include, but is not limited, to personal computers, workstations

25 or mainframes. The processor may be a general purpose processor or microprocessor or a specialized processor executing programs located in RAM memory. The programs may be placed in RAM from a storage device, such as a disk or pre-programmed ROM memory. The RAM memory in one embodiment is used both for data storage and program execution. The computer system also embraces systems where the processor and memory

30 reside in different physical entities but which are in electrical communication by means of a network.

In an alternative embodiment, the program screens for a mutation selected from, in one embodiment, in one embodiment, rT38K, rR55H and rA181V; in another embodiment include rS/T78S, rV80L, rN/S118N, rN/K139K, rE142V, rA/T181A rI204M, 5 rQ/P/S/Stop215S, rE/K218E, rN/H238H and rY245H; in yet another embodiment include rN238T; and yet another embodiment include rI122V and rA181T; in yet another embodiment include rL180M, rA/V200V, rM204V, rV214A, rH237H/P, rV253G, in yet another embodiment include rT128N and rN236T, in yet another embodiment include tL180M, rM204V rQ215S, in yet another embodiment include 10 rT128S rL180M, rM204V and rQ215S, in yet another embodiment include rI80L, rI204M, rN238T, in yet another embodiment include rN238T/A, in yet another embodiment include rI187V, in yet another embodiment include rN123N/I rS135Y, rV214A/V and rQ215Q/P/Stop/S, or a combination thereof or an equivalent mutation; in a further embodiment, sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F, in another 15 embodiment include sF55S, sC/Stop69C, sC/Y76Y, sU/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment 20 include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, in yet another embodiment include sL42R, sQ102Q/R, sT115T/S, sS207S/R, sL215R and sL216Stop, or a combination thereof or an equivalent mutation.

25

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1*Overlapping genome of HBV*

The overlapping genome of HBV is represented in Figure 1. The gene encoding DNA
 5 polymerase (P), overlaps the viral envelope genes, Pre-S1 and Pre-S2, and partially
 overlaps the X and core (C) genes. The HBV envelope comprises small, middle and large
 proteins HBV surface antigens. The large protein component is referred to as the HBV
 surface antigen (HBsAg) and is encoded by the S gene sequence. The Pre-S1 and Pre-S2
 gene sequences encode the other envelope components.

10

EXAMPLE 2*Patients on ADV Treatment and Analysis of HBV DNA*

Patient A: During ADV treatment, unique HBV mutations were detected by
 15 sequencing (Table 4). This includes the unique mutation at rtT38K, and rtA181V. A
 number of other changes were also detected in the polymerase rtR55H and in the
 overlapping envelope gene (Table 4, Figures 4, 5 and 6). The changes in the HBsAg
 include sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F. These unique changes were
 compared to reference sequences from each of the seven genotypes A-G as well as a
 20 consensus sequence from pretreatment samples to determine unique changes

Patient B: The HBV mutations during ADV treatment are listed in Table 5 and Figures
 7, 8, and 9. The unique changes in the rt region of the HBV DNA polymerase include
 rtY245H. Other changes in the HBV polymerase while on ADV treatment include
 25 rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A, rtI204M,
 rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H. The changes in the HBsAg while on ADV
 treatment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y,
 sStop/W172W, sStop/W196W, sS/R207R.

30 Patient C: The HBV mutations prior to ADV treatment and during ADV treatment are
 listed in Table 6 and Figures 10, 11 and 12. The unique changes in the rt region of the

HBV DNA polymerase while on ADV treatment include rtN238T. The unique changes in the HBsAg include sV14A, sL95W, sV96G, and sI208T/I

Patient D: The HBV mutations during ADV treatment is listed in Table 7 and Figures 13, 14 and 15. The unique changes in the HBV DNA polymerase include rtI122V and rtA181T. The unique changes in the surface include sT47A and sW172stop.

Patient E. This patient was previously treated with lamivudine and selected the unique mutations rtH237H/P while on LMV. This patient did not respond to ADV treatment. Changes in the polymerase on ADV treatment include rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P and rtV253G. The unique changes in the surface include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M. NB: Patient on TFV and responded. Changes on ADV listed in Table 8 and Figures 16, 17 and 18.

Patient F: Unique changes during ADV treatment include the polymerase mutations at rtN238T and envelope mutations at sS53L. Changes on ADV listed in Table 9 and Figures 19, 20 and 21.

Patient G: Unique mutations while on ADV treatment include changes in the polymerase rtT128N and rtN236T and a change in envelope sP120T. Changes on ADV listed in Table 10 and Figures 22, 23 and 24.

Patient H: Mutations in the polymerase gene while on ADV treatment include rtL180M, rtM204V, rtQ215S. Changes in envelope gene includes N40S, sS207R. Changes on ADV listed in Table 11 and Figures 25, 26 and 27.

Patient I: Mutations in the polymerase gene while on ADV treatment include rtT128S, rtL180M, rtM204V and rtQ215S, while mutations in the envelope gene included sQ101R, sI195M, sS207R. Changes on ADV listed in Table 12 and Figures 28, 29 and 30.

Patient J: Mutations in the polymerase gene included rtI80L, rtI204M, rtN238T and mutations in envelope sL95W and sL196W during ADV treatment. Changes on ADV listed in Table 13 and Figures 31, 32 and 33.

- 5 Patient K: Mutations in the polymerase gene at rtN238T/A was detected during Adv treatment. No changes in envelope were detected during treatment. Changes on ADV listed in Table 14 and Figures 34, 35 and 36.

- 10 Patient L: Mutations in the polymerase gene at rtI187V was detected during ADV treatment. A mutation in the envelope gene at sV14A was also detected. Changes on ADV listed in Table 15 and Figures 37, 38 and 39.

- 15 Patient M is an HIV HBV co-infected patient treated with tenofovir and lamivudine as part of anti-retroviral therapy for HIV. These two agents are also useful in the treatment of HBV. This patient has not been treated with ADV. Three sequential samples were sequenced while the patient was on antiviral treatment: In one sample there were unique changes in the polymerase at rtN123N/I, rtS135Y, and rtQ215Q/P/ stop/S. Unique changes in the envelope gene were detected at sL42R, sQ102Q/R, sT115T/S. These changes are listed in Table 16 and Figures 40, 41 and 42. The remaining two samples were identical to
- 20 each other in the polymerase and envelope 9 (HBsAg) gene. Unique changes in the polymerase were detected at rtS135Y and rtV214A/V, and rtS207S/R and unique changes in the envelope gene were detected at sQ102Q/R, sL215R and sL216Stop while on TFV and LMV treatment. These changes are listed in Table 16 and Figures 43, 44 and 45.

- 25 The changes at rtQ215Q/P/Stop/S and rtV214A/V have been previously detected in ADV treated patients. Thus, these mutations are important mutations and may result in potential cross-resistance to both ADV and TFV.

EXAMPLE 3

Detection of Viral Markers

Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), anti-HBe and
5 hepatitis B core antigen (HBcAg) specific IgG and IgM were measured using
commercially available immunoassays (Abbott Laboratories, North Chicago, IL, USA).
Hepatitis B viral DNA levels were measured using a capture hybridization assay according
to the manufacturer's directions (Digene Hybrid Capture II, Digene Diagnostics Inc.,
Beltsville, MD). The manufacturers stated cut-off for detecting HBV viremia in clinical
10 specimens was 0.7×10^6 copies/ml or 2.5 pg/ml, [Hendricks *et al.*, *Am J Clin Pathol* 104:
537-46, 1995]. HBV DNA levels can also be quantitated using other commercial kits such
as Cobas amplification HBV monitor kit (Roche).

EXAMPLE 4

Sequencing of HBV DNA

15 HBV DNA was extracted from 100 μ l of serum as described previously by Aye *et al.*, *J.*
Hepatol. 26: 1148-1153, 1997. Oligonucleotides were synthesized by Geneworks,
Adelaide, Australia. Amplification of the HBV polymerase gene has been described by
20 Aye *et al.*, 1997, *supra*.

The specific amplified products were purified using PCR purification columns from MO
BIO Laboratories Inc (La Jolla, CA) and directly sequenced using Big Dye terminator
Cycle sequencing Ready Reaction Kit (Perkin Elmer, Cetus Norwalk, CT). The PCR
25 primers were used as sequencing primers, OS1 5'-GCC TCA TTT TGT GGG TCA CCA
TA-3' (nt 1408-1430) [SEQ ID NO:3], TTA3 5'-AAA TTC GCA GTC CCC AAA-
3'(nt2128-2145) [SEQ ID NO:4], JM 5'-TTG GGG TGG AGC CCT CAG GCT -
3'(nt1676-1696) [SEQ ID NO:5], TTA4 5'-GAA AAT TGG TAA CAG CGG -3' (nt 2615-
2632) [SEQ ID NO:6], OS2 5' TCT CTG ACA TAC TTT CCA AT 3' (nt 2798-2817)
30 [SEQ ID NO:7], to sequence the internal regions of the PCR products.

EXAMPLE 5***Adefovir Dipivoxil (ADV)***

5 ADV (formerly Bis-pom PMEA)) is a potent inhibitor of HBV replication. The structure of ADV is shown in Figure 2 and its synthesis is described by Benzaria *et al.*, *J Med Chem.* 39: 4958-4965, 1996).

Those skilled in the art will appreciate that the invention described herein is susceptible to
10 variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features

15

Table 4: Patient A HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV viral load	HBV RT Mutations	HBsAg Mutations
ADV	22/10/02	8.77E+06	-	sT47A sT131T/I
ADV	14/01/03	1.21 E+09	-	-
ADV	8/7/03	7.92 E+07	rtT38K rtA181V	sL173F

Table 5: Patient B RT and Polymerase mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	13/02/03	rtV80L - rtN118N/S rtN139N/K - rtV142E rtA181A/T - rtI204M rtQ/P/S/Stop215Q rtE218K/E rtN238N/H	- sC76Y/C sI110V/I - sN131N/T sY134N sW172Stop/W sStop196W sR/S207S
ADV	21/03/03	rtS78T/S rtV80L - rtN118N/S rtN139N/K - rtV142E rtA181A/T - rtI204M rtQ215 Q/P/S/Stop - rtE218K/E rtN238N/H	sS55F sC69Stop - sC76Y/C sI110V/I sN131N/T - sY134N sW172Stop/W sStop/W196W sS207S/R

ADV	22/07/03	rS/T78S rV80L rN/S118N rN/K139K - rE142V rA/T181A - rI204M rQ/P/S/Stop21 r5SE/K218E rN/H238H rY245H	sF55S sC/Stop69C sC/Y76Y sI/V110I sN/T131N - sN134Y sStop/W172W sStop/W196W sS/R207R
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Table 6: Patient C HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	18/08/03	rN238T	sV14A sL95W sV96G sI208T/I

Table 7: Patient D HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	20/08/03	rI122V rA181T	sT47A sW172Stop

Table 8: Patient E HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
LMV	23/05/02	rtL180M rtA200V/A rtM204V rtV214A rtP237H	- sL192L/F sI195M
ADV	17/07/03	- - rtL180M rtA/V200V rtM204V rtV214A rtH237H/P rtV253G	PreS2 T6S sT47A sP62L sL/F192F sI195M

Table 9: Patient F HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	16/10/03	rtN238T	sS53L

Table 10: Patient G HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	12/11/03	rtT128N rtN236T	sP120T

Table 11: Patient H HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	05/11/03	rtL180M rtM204V rtQ215S	sN40S sI195M sS207R

Table 12: Patient I HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	05/02/03	- rtT128S rtL180M rtM204V rtQ215S	sQ101R - - sI195M - sS207R

Table 13: Patient J HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	18/11/03	rtI80L - rtI204M rtN238T	- sL95W sL196W -

Table 14: Patient K HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	31/03/03	rtN238T/A	No changes

Table 15: Patient L HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	17/09/03	rtI187V	sV14A

Table 16: Patient M HBV Polymerase and envelope mutations detected during TFV and LMV therapy

Treatment	Date	Viral load copie/ml	HBV RT Mutations	HBsAg Mutations
TFV LMV	18/06/04	2.76E+06	- - - rtN123N/I rtS135Y rtQ215Q/P/stop/S	sL42R, sQ102Q/R, sT115T/S sS207S/R
TFV LMV	9/7/04	4.31E+07	- rtS135Y rtV214A/V - -	sQ102Q/R - - sL215R sL216 ⁺ .
TFV LMV	22/08/04	>1.00E+08	- rtS135Y rtV214A/V - -	sQ102Q/R. - - sL215R sL216 ⁺ .

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